US Army Corps
of Engineers
Construction Engineering
Research Laboratories

USACERL Special Report 98/56
April 1998

The Incorporation of Cyanobacteria Into Starch Pellets and Determination of Escapability Rates For Use in Land Rehabilitation

by
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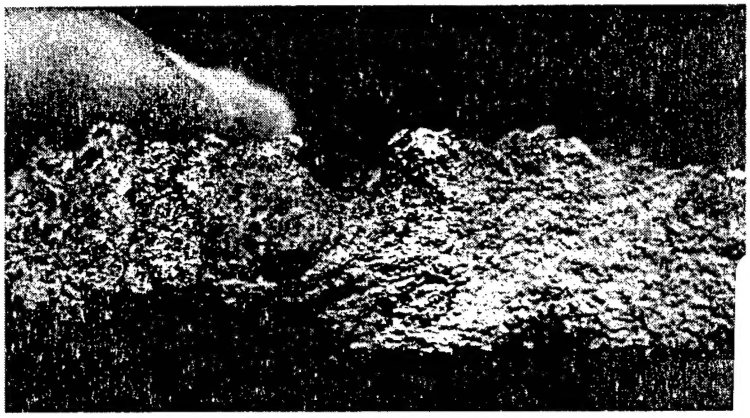

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave Blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	April 1998	Final

4. TITLE AND SUBTITLE
The Incorporation of Cyanobacteria Into Starch Pellets and Determination of Escapability Rates For Use in Land Rehabilitation

5. FUNDING NUMBERS
4A162720
BT25
LLJ17

6. AUTHOR(S)
Gwyn L. Howard and Steven D. Warren

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
U.S. Army Construction Engineering Research Laboratories (USACERL)
P.O. Box 9005
Champaign, IL 61826-9005

8. PERFORMING ORGANIZATION REPORT NUMBER
SR 98/56

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Department of the Army, Assistant Chief of Staff for Installation Management
ATTN: DAIM-ED-R
600 Army Pentagon
Washington, DC 20310-0600

Commander, U.S. Army Environmental Center
ATTN: SFIM-AEC-EQN
Aberdeen Proving Ground, MD 21010-5401

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES
Copies are available from the National Technical Information Service, 5285 Port Royal Road, Springfield, VA 22161.

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution is unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 words)

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14. SUBJECT TERMS
Arid lands
Land management
Land rehabilitation
Soil stabilization
Desert ecology

15. NUMBER OF PAGES
26

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	SAR

Foreword

This study was funded by Headquarters, Department of the Army, Assistant Chief of Staff for Installation Management, Project 4A161102BT25, "Environmental Research—Corps of Engineers"; as part of Work Unit LLJ17, "Cryptogamic Species for Training Land Rehabilitation."

The work was performed by the Resource Mitigation and Protection Division (LL-R) of the Land Management Laboratory (LL), U.S. Army Construction Engineering Research Laboratories (USACERL) as partial fulfillment of the requirements for the degree of Master of Science in Forestry at the University of Illinois at Urbana-Champaign. The University of Illinois advisor was Dr. Richard E. Warner. The USACERL principal investigator was Dr. Steven D. Warren. Robert E. Riggins is Chief, LL-R. Dr. William D. Severinghaus is Operations Chief, CECER-LL. The USACERL technical editor was Gloria J. Wienke, Technical Resources.

Special recognition is due to Dr. Jeffrey R. Johansen, Department of Biology, John Carroll University, University Heights, Ohio; and Dr. L.M. O'Flaherty, Department of Biology, Western Illinois University, Macomb, who served on the graduate committee along with Dr. Warner and Dr. Warren. Dr. Dick L. Gebhart and Thomas A. Hale, both of USACERL-LL-R, provided valuable editorial and statistical assistance.

COL James A. Walter is Commander of USACERL and Dr. Michael J. O'Connor is Director.

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1 Introduction

Background

Accelerated soil erosion by wind and water following anthropogenic disturbance is a problem affecting most ecosystems around the world. It is especially problematic with regard to the environmental health and stability of Army training lands. Degradation of the Army's natural resources results in an overall negative impact on training. Soil erosion may create trails that are impassable for tactical vehicles. Siltation of waterways and retention ponds is increased; flooding becomes a concern. Fugitive dust jeopardizes troop safety, vehicle longevity, and mission effectiveness.

Army land managers are often faced with the difficult challenge of rehabilitating disturbed soils. Revegetation of disturbed areas with native plants is often the most economically feasible and ecologically palatable land rehabilitation measure. However, in semi-arid and arid climates, vascular plants are naturally sparse and revegetation with these species is difficult due to low rainfall, erratic spatial and temporal precipitation patterns, and dramatic daily and seasonal temperature fluctuations. In these areas, soil microphytes (cyanobacteria, algae, lichens, mosses, etc.) have evolved to fill the ecological roles of soil stabilization and nutrient cycling.

In undisturbed desert environments, the primary ground cover (up to 70 percent) is often an association of microphytic organisms sometimes referred to as cryptogamic or biological crusts (Belnap and Gardner 1993). Some researchers believe cryptogamic crusts are critical components of semi-arid and arid ecosystems in terms of soil stability, nutrient cycling, and biodiversity (Evans and Ehleringer 1993; Harper and Marble 1988; Johansen 1993; Kleiner and Harper 1972; Metting 1991; St. Clair and Johansen, 1993; and Starks, Shubert, and Trainor 1981).

Cryptogamic species tend to be early successional or pioneer species in many ecosystems, and become more dominant as later successional species along a gradient of increasing aridity. The use of these organisms as combatants of soil erosion, while suggested by various authors (e.g., Ashley and Rushforth 1984; Belnap 1993; Campbell, Seeler, and Golubic 1989; Knutsen and Metting 1991; St. Clair, Johansen, and Webb 1986), remains a rather unexplored and novel approach in the reclamation, rehabilitation, and stabilization of disturbed soils. The potential

role of these naturally occurring cryptobiotic lifeforms in the reclamation of disturbed sites merits attention.

Objective

The objective of this study was to incorporate cyanobacteria into a pelletized starch matrix for use as a soil inoculant in arid land rehabilitation.

Approach

Two common terrestrial cyanobacteria, *Nostoc punctiforme* and *Microcoleus vaginatus,* were pelletized, applied to a sand substrate, and monitored over an 8-week period (July to August 1996). Escapability and viability of the pelletized cyanobacteria were tested using both chlorophyll *a* extraction and epifluorescence microscopy.

Results

Results were poor and indicated this approach would be ineffective in the field. Direct counts of populations and mean levels of chlorophyll *a* in both species steadily declined during the 8-week period. It is hypothesized the lack of success may be attributed to several factors, primarily desiccation of the organisms during the pelletizing process.

Scope

The future applications of this project include an array of practical uses for U.S. Army installations including roadside stabilization, rehabilitation and revegetation of eroded lands, and conservation of existing habitat.

Mode of Technology Transfer

It is recommended the information in this report be used by installation land managers and training managers while planning approaches to implement rehabilitation and revegetation of disturbed areas in arid and semi-arid lands.

2 Ecological Roles of Cryptogamic Crusts

Cryptogams act as a natural soil stabilizing and conditioning agent. Fritsch (1907) discusses the role blue-green algae play in plant succession. He concludes that algal coverings serve a vital purpose as pioneers of denuded lands. They aid in the nurturing of seedlings by retaining and regulating the moisture in the soil matrix. The gelatinous covering aids in the establishment of seedlings while helping stabilize the soil in which they are developing. A recent study (Belnap 1993) found that the inoculation of barren soils with cyanobacteria greatly improved the recovery rate of exposed soils. The cyanobacteria inoculants hastened and supported the invasion of other cryptogams such as lichens, algae, and mosses.

Cryptogams improve soil structure and nutrient availability. Moss and lichen constituents aid in soil stabilization by covering disturbed surfaces with vegetation and penetrating the soil with rooting structures (Anderson, Harper, and Holmgren 1982). Algae and lichens have been found to be important pioneers in secondary succession on eroded land (Booth 1941). Booth concluded that cryptogams aid in the genesis of organic matter, increase nutrient availability and water infiltration, fix nitrogen, and increase soil stabilization. Following the invasion of the soil matrix by filaments, improvement in soil structure occurs (Metting and Rayburn 1983). Through incorporation of organic carbon via photosynthesis, and the contribution of nitrogen through nitrogen fixation, some cyanobacteria are primary benefactors of improved soil fertility. Both organic and inorganic forms of nitrogen can be increased by the nitrogen fixation/decomposition cycles of cyanobacteria.

In U.S. deserts, it is thought that nitrogen fixation by cyanobacteria is the primary avenue by which desert soils obtain nitrogen (Evans and Ehleringer 1993; Fuller, Cameron, and Raica 1960; MacGregor and Johnson 1971; Shields and Durrell 1964). Regardless of the mechanism, cryptogamic crusts are critical contributors to the nitrogen pool in desert soils. Cryptogamic crusts also act as a reservoir for various essential nutrients, making them available to higher plants (Harper and Pendleton 1993). For these reasons, they are important contributors in both mature and developing soils.

As a general rule, cryptogams increase infiltration and decrease evapotranspiration. Water infiltration and percolation is greatly increased by the cryptogamic micro-environment (Booth 1941; Brotherson and Rushforth 1983; Eldridge 1983; Fletcher

and Martin 1948; Schere et al. 1984). By increasing infiltration, cryptogamic crusts assist plants in several ways. First, more total water is available for seedling establishment and growth. Second, evaporation is reduced and moisture is retained more successfully due to the dense, protective layering of the cryptogamic crust. Lastly, with less water movement on the surface, less soil is removed by water movement, thereby reducing soil erosion. By being an integral part of the colonial synusium, cryptogams play an important role in primary and secondary plant community succession.

Cryptogams form a natural and protective soil covering. They play a vital part in the binding of soil surface particles, thereby helping to reduce wind and water erosion. Algal and fungal filaments intertwine with soil particles to produce a stable soil surface tapestry (Durrell and Shields 1961). Extracellular polysaccharide secretions from the cyanobacterial filaments also bind soil particles, facilitating aggregation of soil particles (Barclay and Lewin 1985; Bond and Harris 1964; Fletcher and Martin 1948). This process helps form a stabilized soil crust that holds down the soil, prevents further erosion, and creates, by the death and decay of cryptogamic organisms, a nutrient layer able to support the establishment of higher plants.

Cryptogams also disrupt air movement and reduce wind erosion. Wind currents in the microenvironment of the cryptogamic crusts tend to be broken up by irregularities of the crust (Brady 1974). Soil movement by wind is greatly reduced, thereby lessening the potential for soil erosion. This microenvironment aids in the reduction of wind erosion and increases the probability for site stabilization through enhanced seed germination and plant establishment (St. Clair et al. 1984). Within this microenvironment, algal propagules are spread and dispersed via air currents. The microclimate provided by the irregular surface area aids in collecting organic matter for the seedbed and improved water availability to plants.

3 Uses of Cryptogams in Land Rehabilitation

Cryptogams, especially cyanobacteria, are a contributor to arid land stability. Cryptogamic organisms are ubiquitous and can be found firmly established in environments as diverse as the Mojave desert and the Arctic tundra (Friedmann and Galun 1974; Harper and Marble 1988; West 1990). They can lie dormant without water for as long as 83 years and are able to withstand temperature extremes ranging from -192°C to 100°C (Cameron and Blank 1966; Metting 1981). Their ability to survive and flourish on various substrates and in microenvironments in all latitudes on earth is especially useful in creating a foundation for succession of higher plants.

With a well-developed and firmly established cryptogamic crust, many species are capable of revegetating otherwise barren soils. Although it has been observed in one study that germination of a broadcast sown grass seed can be decreased by an established cryptogamic crust as compared with barren soil (McIlvanie 1942), it should be noted that once established, seedlings grow more rapidly in the presence of a cryptogamic crust (Metting 1981). Cracks in the crust are ideal microsites in which seeds of vascular plants can lodge and grow (St. Clair et al. 1984).

Controlled studies on the influence of cryptogamic crusts and their use in the rehabilitation and stabilization of disturbed soils are limited. However, from initial observations and detailed records of those having studied cryptogamic activity, it seems very plausible to pursue the practical uses of cryptogams. Creating a pellet that is easily biodegraded, dispersed simultaneously with grass seed, and packaged, stored, and transported to field sites would be beneficial for many Army land restoration projects.

Previously, studies had been conducted using calcium alginate as an entrapment matrix for cyanobacteria in the development of dry pellets (Buttars et al. 1997; Johansen et al. in review). Although the cyanobacteria were viable after encapsulation, they were not able to easily escape into the surrounding environment through the intact pellets; grinding was necessary. After speaking with several researchers and conducting a literature review, a potential alternative for developing a suitable matrix was found—the Dunkle and Shasha method of starch encapsulation (Dunkle and Shasha 1988). This method was found to be suitable for a variety of other

microorganisms, including bacteria (Chandler, McGuire, and Shasha 1995; McGuire and Shasha 1994, 1995). Originally developed to form an affordable biodegradable herbicide carrier, this method offered several attractive features, including the simplicity and ease of incorporating living organisms into the matrix, the gentle physical nature of starch, the abundance of inexpensive cornstarch, and the biodegradability of the pellets. Initial trials indicated the incorporation of cyanobacteria into a starch matrix would be successful. The current project examined rates of escapability and longevity of cyanobacteria incorporated into a starch matrix.

4 Materials and Methods

Cyanobacteria for this study, *Nostoc punctiforme* and *Microcoleus vaginatus*, were isolated from the Dugway Proving Grounds in Utah and cultured by Dr. Jeff Johansen at John Carroll University, University Heights, OH. Cultures were grown in Z-8 media with continuous aeration and solution circulation to optimize growing conditions. Cyanobacteria were harvested when density levels reached 5 g/L or higher. Upon arrival at USACERL, an epifluorescence survey was conducted to ensure the cyanobacteria were still viable.

Pellets were processed according to the method developed by Dunkle and Shasha (1988). One part of pregelatinized cornstarch (25 g) was added to 1 part water (25 mL) containing 2.5 g/L cyanobacteria and quickly mixed together. The mixture was then allowed to set up at room temperature for 30 minutes to provide adequate time for retrogradation to occur. Retrogradation of the starch molecules results in a matrix that entraps active agents within pockets of the reassociated molecules (McGuire and Shasha, 1995). At the end of 30 minutes, the mass was placed into a blender to be chopped into discreet granules that were then spread into a sterilized pan, covered with paper towels, and allowed to air-dry for 24 hours. The resulting pellets were stored in sterilized glass containers for 2 weeks prior to distribution onto the soil surfaces. Before placement onto the soil surface, pellets were sifted through sterilized sieves to obtain uniform pellets of a 100 mesh size.

An experimental unit consisted of one 25 cm x 25 cm soil tray within which 100 sampling areas existed in a centrally located 20 cm x 20 cm grid; the centrality of the sampling area lessened the possibility of any edge effects on the experimental units. Trays were filled 2 cm deep with a fine grain silica sand and a 10 x 10 grid was marked off along the sides of the soil trays for a total of 100 sampling areas (8 cm^3 each) within each replicate. Using a complete randomized block design, 35 g of sifted pellets were uniformly applied to the central grid on the sterilized soil trays (4 replications/species and 1 control/species). Replicates were placed in the controlled atmosphere of a EGC model G-10 growth chamber at USACERL for the duration of the study. Early springtime conditions were simulated; the steady rise and fall of daytime temperatures ranging from 20°C to 25°C, 12:12 hr L:D photoperiods with light intensities ramping from 0 to100 $\mu E\ m^{-2}s^{-1}$, and humidity of 40 to 50 percent at all times. Soil trays within and between blocks of replications

were systematically rotated on a weekly basis to eliminate errors due to potential uneven light distribution. The experimental units were incubated for 8 weeks (July to August 1996).

After the first 2 weeks of the study, soil samples (2 cm^3) were gathered weekly over the course of 8 weeks for analyses. Using a random number generator, coordinates for weekly sampling cores were determined prior to the initiation of the study. Three samples were taken weekly from each replicate for chlorophyll *a* analysis. This sampling scheme was repeated for the epifluorescence analysis, except that the samples were combined to form a single composite sample for each replicate. Escapability and viability were determined by using epifluorescence microscopy to determine numbers and volumes of cyanobacteria present and chlorophyll *a* extraction methods to estimate biomass. Initial values for chlorophyll *a* and epifluorescence within a 1.5-g sample size of pellets were established to serve as a baseline. Results were analyzed with the SYSTAT statistical software using a student's t-test to determine differences in variance between species over the course of the study.

Chlorophyll *a*

After the weekly soil samples were collected, they were placed in individual bags to be crushed by a mortar and pestle to create a homogenous sample. This sample was allowed to dry overnight in a dark place. The following day, 1.5-g of soil was weighed out from each dried 2-cm^3 sample. Chlorophyll *a* was extracted from the soil samples with dimethyl sulfoxide (DMSO) after a method established by Ronen and Galun (1984). To prevent the degradation of chlorophyll *a* during the extraction process, all of the steps were performed in the presence of minimal light. Ten mL of DMSO was added to each 1.5-g soil sample and the mixture was then placed in a centrifuge tube and shaken. After combining the soil with the DMSO, samples were heated in an oven at 65°C. At 30 minutes the samples were removed from the oven in dim light, shaken to thoroughly homogenize the DMSO in the sample, and then placed back into the oven for another 30 minutes. Samples were then removed from the oven and allowed to cool in dim light. After cooling, samples were centrifuged at 2,500 rpm for 10 minutes. The resultant supernatant was poured into a glass test tube for further analysis.

Each sample was analyzed using a Varian Cary 3G UV/VIS spectrophotometer at wavelengths of both 665 nm and 730 nm. Using a spectrophotometer cuvette with beam length of 1 cm, values for the absorbance peak of chlorophyll *a* and the correction factor for turbidity were determined. Chlorophyll *a* has an absorbance

peak at 665 nm. The 730 nm peak is used as a correction figure for turbidity in the calculation of total chlorophyll *a* extraction. After recording the absorbance values at both peaks, the samples were acidified with 20-µL of HCl for 5 minutes and mixed thoroughly. The absorbance values were then recorded again at both peaks.

Lorenzen (1967) gives a ratio of 1.7 in the values of before and after acidification used in the following calculations for the reduction of chlorophyll *a* from the original absorbance values. Vollenweider (1969) introduced the equation to determine chlorophyll *a* levels with regard to correction values for phaeophytin. To determine micrograms (µg) chlorophyll *a* per cubic centimeter of soil, the following equation was used:

$$\text{Chlorophyll } a = 89.3[(665_{before}\text{-}730_{before})\text{-}(665_{after}\text{-}730_{after})]$$

This equation assumes 1.5-g of dry soil and a spectrophotometer cuvette with a beam length of 1 cm.

Epifluorescence

The epifluorescence method used for the enumeration and determination of soil algae biovolume in this study was based on the original methods of Tchan (1952) with modifications by Johansen and Rushforth (1985). Each replicate soil tray yielded three 2-cm^3 soil samples, which were combined to form a composite sample. Fifteen ml of distilled water was combined with each composite and vortexed. One ml of supernatant was then transferred by pipette into a tissue grinder (15 ml, composed of a steel plunger rod and a plastic head) and crushed. The homogenized sample was then transferred by pipette onto an American Optical 0.1-mm deep bright line hemacytometer slide and examined using an Olympus BH2 microscope with an RFC (reflected light fluorescence) using the blue excitation filter. Using a dark field, the fluorescing (living) algae were examined at 400x magnification across the length of the slide for 20 transects. Only fluorescing algal cells were measured and frequencies recorded for determining the biovolume of soil algae for each composite. For *Nostoc*, a colonial cyanobacteria, biovolume was estimated by measuring observed length vs. width of colonies, calculating volume of an oval using these data, and then recording frequencies. Biovolume of *Microcoleus*, a filamentous cyanobacteria, was ascertained by determining the average diameter of a filament, measuring the length of each filament, calculating the volume of a cylinder, and recording frequencies. The following formulae were used in determining the frequency and biovolume of cyanobacteria in each soil sample.

$$\text{Frequency} = \frac{(\#\ \text{filaments observed})\ (\text{amount of dilutant})}{(\#\ \text{transects})\ (\text{volume/transect})\ (\text{amount of sample})}$$

$$\text{Biovolume} = \frac{(\text{total biovolume})\ (\text{amount of dilutant})}{(\#\ \text{transects})\ (\text{volume/transect})\ (\text{amount of sample})}$$

With the Olympus BH-2 at the University of Illinois, the volume per transect is: (0.1-mm deep) (6-mm long) (0.380-mm high) (0.001-ml/mm^3) = 0.000228-ml. The value of biovolume is equal to (total length of a filament) (πr^2) where r is ½ the cell width, which can then be placed into the formula for Biovolume. The amount of dilutant is 5 mL; amount of the sample is 3 cm^2.

5 Results and Discussion

Although the initial studies indicated the cornstarch pellets would provide a suitable suspension matrix for the cyanobacteria, results from this study were not as encouraging. Most traces of cyanobacteria in both sets of replicates quickly disappeared during the first 2 to 4 weeks of the study. Equipment failure precluded the analysis of samples from week 3.

Weekly and cumulative chlorophyll *a* contents were not significantly different between species ($P = 0.05$). Chlorophyll *a* content in *Microcoleus* declined from an initial level of 0.1036-μg/g to 0.0191-μg/g; *Nostoc* declined from 0.156-μg/g to 0-μg/g. The slower rate of decline in the *Microcoleus* replicates may be due to the delay encountered while preparing the pellets for each species. *Nostoc* was readily available in the lab for pelletizing while preparation of the *Microcoleus* pellets was delayed by a week due to unavailability of ample supplies of stock solution. Baseline levels of both species were established immediately prior to dispersal onto the soil; 1.5 g of pellets yielded chlorophyll *a* contents of 2.197-μg/g of pellets for *Microcoleus* and 0.750-μg/g of pellets for *Nostoc*. This observation lends validity to the hypothesis that desiccation of the organisms during the pelletizing process may have been a prime reason for lack of success in this study.

Epifluorescence counts uncovered only a single *Nostoc* colony and a single *Microcoleus* filament during the first sampling week of the study; no other microorganisms were observed for the remainder of the experiment. Frequency and biovolume for *Nostoc* were 110/cm^3 and 458.9-μg/cm^3, respectively; *Microcoleus* was 110/cm^3 and 123.9-μg/cm^3. Mean values were used to plot chlorophyll *a* levels in both species (Figure 1).

Differences in soil cohesiveness during the sampling process were also observed. During the course of collecting weekly samples, it was noted that the *Microcoleus* samples consistently differed from those of *Nostoc*. The 2-cm^3 samples were far easier to extract from the soil tray. Despite the fact that equal amounts of water were added to each replicate, *Microcoleus* samples appeared to consistently retain more overall soil moisture than those of *Nostoc*. Also, *Microcoleus* samples were notably and invariably more intact than those of *Nostoc*; samples came out easily and remained in the form of the original cube. Lastly, the *Microcoleus* pellets

themselves seemed more stable and amalgamated than the *Nostoc* pellets. The variance may be due to the difference between the processing and/or distributing of the pellets. As the *Nostoc* pellets were processed a week before the *Microcoleus* pellets, alterations within the chemical bonds of the starch matrix may have occurred during that time. Discrepancies may also be attributed to the differences between the species and their growth patterns. Considering the nature of *Microcoleus*, this is consistent with observations that *Microcoleus* filaments tend to weave together soil particles (Belnap and Gardner 1993). *Microcoleus* is also motile and can move up to 5 cm in 24 hours (Belnap, Harper, and Warren 1994) thereby depositing exuded polysaccharides throughout the soil matrix, whereas *Nostoc* is also filamentous, but non-motile and less likely to leave widespread paths of mucilage. These observations are of note and could lead to further studies.

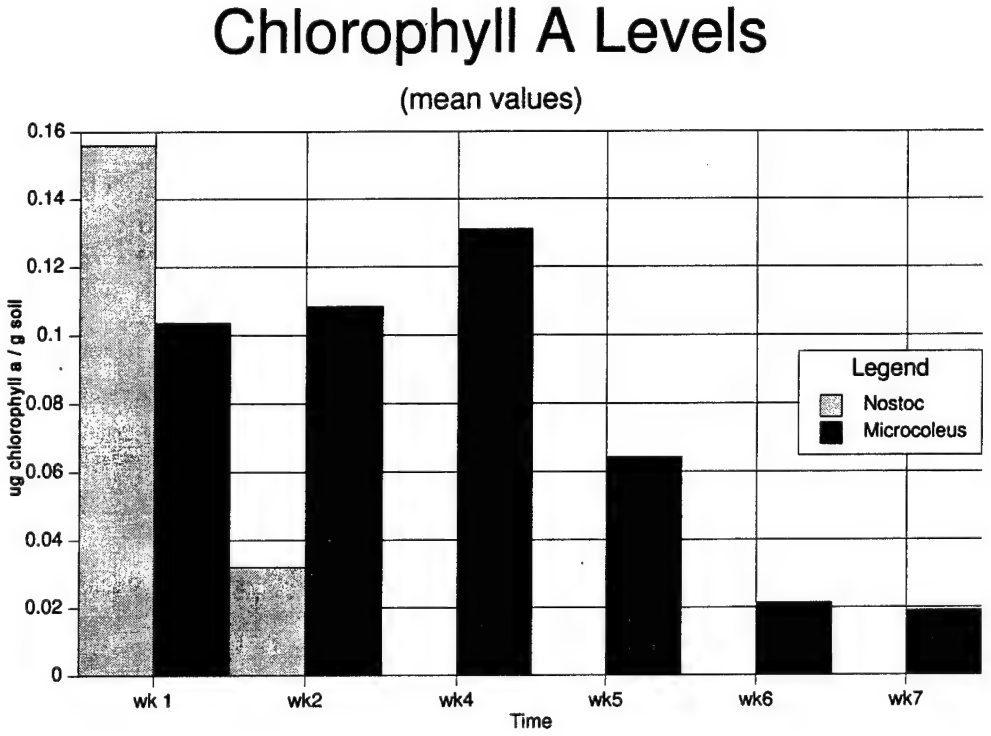

Figure 1. Sampling was started (wk1) at the beginning of the second full week into the study.

	Week 1	Week 2	Week 4	Week 5	Week 6	Week 7
nostoc	0.156	0.0321	0	0	0	0
micro	0.1036	0.1084	0.131	0.0643	0.0214	0.0191

6 Conclusion and Recommendations

Although the cyanobacteria in this study did not perform as well in the cornstarch pellets as had been expected, it did shed some light onto other possible avenues to pursue in future studies. It is believed there may be a critical mass beyond which the cyanobacteria may survive this method of processing. The cyanobacteria may have been out-competed by the starch granules for the available hygroscopic water within the molecular lattice of the pellets. It may be possible that with higher levels of cyanobacteria in the starch matrix, the algae will clump together and protect or conserve vital water enabling some of the population to survive and escape the pellets. Previous experiments with alginate pellets indicated 2.5 g/L of inoculant are successful in providing sufficient amounts of inoculants to barren soils. With larger amounts of inoculant, there may exist a greater potential for survival and escape. The same effect may be induced by using different species that may be more resilient or resistant to water losses. Currently, studies have been initiated to pursue the use of tapioca pellets as a possible method of inoculation. The pellets are ready-made, uniform in size, abundant, and have the capacity to form chemical bonds with the naturally occurring polysaccharides exuded by cyanobacteria. Plans are being made to research the use of wastes from commercial food processing plants such as broken grains of rice, corn, soybeans, and alfalfa as inoculant carriers. Additional research plans include incorporating cyanobacteria into an array of carriers such as clay, sand, grass seed, soil tackifiers, mulch, or other soil amendments.

The problem of desertification is large and has many intricacies yet to be discovered. However, steps are being taken to uncover these mysteries and put them to practical use in the reclamation of endangered ecosystems. The use of soil inoculants is a viable solution in the remediation of some disturbed lands. There also exists the likelihood of making available commercially produced inoculant materials that have a long shelf-life and are easy to handle. Economically, it may be feasible; with regard to long-term stewardship of our natural resources, it may be requisite.

The main objective of this study was to attempt to find a suitable matrix in which to encapsulate native cyanobacteria and reintroduce them to their environment. Although this particular goal was not obtained, valuable and useful information

was gained for the next round of testing. This study helps demonstrate the need for further development of dispersal mechanisms, a better understanding of the intricacies involved in handling cyanobacteria during various processing practices, and the necessity of gaining more knowledge of individual species on the microscopic level. Of course there are no simple answers, but the role desert crust inoculants play in the battle against encroaching desertification seems to be of primary importance at this point in our understanding. Given time, a practical and economically feasible product will be developed to help combat some of the problem areas within semi-arid and arid lands.

References

Anderson, D.C., K.T. Harper, and R.C. Holmgren. 1982. Factors influencing development of cryptogamic soil crusts in Utah deserts. Journal of Range Management 35(2):180-185.

Ashley, J., and S.R. Rushforth. 1984. Growth of soil algae on topsoil and processed oil shale from the Uintah Basin, Utah, U.S.A. Reclamation and Revegetation Research 3: 49-63.

Barclay, W.R., and R.A. Lewin. 1985. Microalgal polysaccharide production for the conditioning of agricultural soils. Plant and Soil 88: 159-169.

Belnap, J. 1993. Recovery rates of cryptobiotic crusts: inoculant use and assessment methods. Great Basin Naturalist 53(1):89-95.

Belnap, J., and J.S. Gardner. 1993. Soil microstructure in soils of the Colorado Plateau: the role of the cyanobacterium *Microcoleus vaginatus*. Great Basin Naturalist 53(1):40-47.

Belnap, J., K.T. Harper, and S.D. Warren. 1994. Surface disturbance of cryptobiotic soil crusts: nitrogenase activity, chlorophyll content, and chlorophyll degradation. Arid Soil Research and Reclamation 8: 1-8.

Bond, R.D., and J.R. Harris. 1964. The influence of the microflora on physical properties of soils. Australian Journal of Soil Research 2: 11-122.

Booth, W.E. 1941. Algae as pioneers in plant succession and their importance in erosion control. Ecology 22(1):38-46.

Brady, N.C. 1974. The nature and properties of soils. 8th ed. Macmillan Publishing Co., Inc., New York. 639 pp.

Brotherson, J.D., and S.R. Rushforth. 1983. Influence of cryptogamic crusts on moisture relationships of soils in Navajo National Monument, Arizona. Great Basin Naturalist 43(1):73-78.

Buttars, S.M., L.L. St. Clair, J.R. Johansen, J.C. Sray, M.C. Payne, B.L. Webb, R.E. Terry, B.K. Pendleton, and S.D. Warren. In review. Pelletized cyanobacterial soil amendments: Laboratory testing for survival, escapability, and nitrogen fixation. Arid Soil Research and Rehabilitation.

Cameron, R.E., and G.B. Blank. 1966. Soil studies – Desert microflora. XI. Desert soil algae survival at extremely low temperatures. Jet Propulsion Laboratory Space Programs Summary 37-37, IV: 174-181.

Campbell, S.E., J.S. Seeler, and S. Golubic. 1989. Desert crust formation and soil stabilization. Arid Soil Research and Rehabilitation 3: 217-228.

Chandler, L.D., M.R. McGuire, and B.S. Shasha. 1995. Evaluation of starch-encapsulated diflubenzuron for use in management of Fall Armyworm (Lepidoptera: Noctuidae) larvae. Journal of Agricultural Entomology 12(1): 33-44.

Dunkle, R.L., and B.S. Shasha. 1988. Starch-encapsulated Bacillus thuringiensis: a potential new method for increasing environmental stability of entomo-pathogens. Environmental Entomology 17: 120-126.

Durrell, L.W., and L.M. Shields. 1961. Characteristics of soil algae relating to crust formation. Transactions of the American Microscopical Society 80:73-79.

Eldridge, D.J. 1993. Cryptogam cover and soil surface condition: effects on hydrology on a semiarid woodland soil. Arid Soil Research and Rehabilitation 7: 203-217.

Evans, R.D., and J.R. Ehleringer. 1993. A break in the nitrogen cycle in aridlands? Evidence from $\delta^{15}N$ of soils. Oecologia 94: 314-317.

Fletcher, J.E., and W.P. Martin. 1948. Some effects of algae and molds in the rain-crust of deserts soils. Ecology 29(1): 95-100.

Friedmann, E.I., and M. Galun. 1974. Desert algae, lichens, and fungi. Pages 165-212 *in* G.W. Brown, ed., *Desert Biology II.* Academic Press, London.

Fritsch, F.E. 1907. The role of algal growth in the colonization of new ground and in the determination of scenery. The Geographical Journal 30:531.

Fuller, W.H., R.E. Cameron, and N. Raica. 1960. Fixation of nitrogen in desert soils by algae. 7th Intern. Congress of Soil Science, Madison, WI, USA. 3:617-624.

Harper, K.T., and J.R. Marble. 1988. A role for nonvascular plants in management of arid and semiarid rangeland, pp. 135-169, *in* P.T. Tueller, ed., *Vegetation Science Applications for Rangeland Analysis and Mangement.* Kluwer Academic Publishers, Dordrecht, The Netherlands.

Harper, K.T., and R.L. Pendleton. 1993. Cyanobacteria and cyanolichens: Can they enhance availability of essential minerals for higher plants? Great Basin Naturalist 53:59-72.

Johansen, J.R. 1993. Cryptogamic crusts of semiarid and arid lands of North America. Journal of Phycology 29:140-147.

Johansen, J.R., and S.R. Rushforth. 1985. Cryptogamic soil crusts: Seasonal variation in algal populations in the Tintic Mountains, Juab County, Utah. Great Basin Naturalist 45(1): 14-21.

Johansen, J.R., L.L. St. Clair, K.A. Kasper, B. Pendleton, and S.D. Warren. In review. Pelletized cyanobacterial soil amendments: development and field testing for use in restoration of arid-land microbiotic soil crusts.

Kleiner, E.F., and K.T. Harper. 1972. Environment and community organization in grasslands of Canyonlands National Park. Ecology 53: 299-309.

Knutsen, G., and B. Metting. 1991. Microalgal mass culture and forced development of biological crusts in arid lands. Pages 487-506 *in* Skujins, J., ed.; *Semiarid Lands and Deserts – Soil Resource and Reclamation*, Marcel Dekker, Inc.

Lorenzen, C.J. 1967. Determination of chlorophyll and pheo-pigments: spectrophotometric equations. Limnology and Oceanography 12: 343-346.

MacGregor, A.N., and D.E. Johnson. 1971. Capacity of desert algal crusts to fix atmospheric nitrogen. Soil Science Society of America Proceedings 35(5): 843-844.

McGuire, M.R., and B.S. Shasha. 1994. Starch encapsulation of microbial pesticides for sustained activity. Pages 71-85 in Doan, W.M, and M.M. Schreiber, eds. Starch-encapsulated pesticides: ARS papers presented at the international Seminar on Research and Development of Controlled-release Formulations of Pesticides, Vienna, Austria, September 6-10, 1993. USDA, ARS, 1995-1.

McGuire, M.R., and B.S. Shasha. 1995. Starch encapsulation of microbial pesticides. Pages 229-237 *in* Hall, F.R.and J.W. Barry, eds; *Biorational Pest Control Agents: Formulation and Delivery*, American Chemical Society.

McIlvanie, S.K. 1942. Grass seedling establishment, and productivity – overgrazed vs. protected range soils. Ecology 23(2): 228-231.

Metting, B. 1981. The systematics and ecology of soil algae. The Botanical Review 47(2):195-312.

Metting, B. 1991. Biological surface features of semiarid lands and deserts. Pages 257-293 *in* J. Skujins, ed., *Semiarid lands and deserts: soil resource and reclamation.* Marcel Dekkert, Inc., New York.

Metting, B., and W.R. Rayburn. 1983. The influence of a microalgal conditioner on selected Washington soils: and empirical study. Soil Science Society of America Journal 47:682-685.

Ronen, R., and M. Galun. 1984. Pigment extraction from lichens with dimethyl sulfoxide (DMSO) and estimation of chlorophyll degradation. Environmental and Experimental Botany 24: 239-245.

Schere, S., A. Ernst, T. Chen, and P. Boger. 1984. Rewetting of drought-resistant blue-green algae: Time course of water uptake and reappearance of respiration, photosynthesis, and nitrogen fixation. Oecologia (Berlin) 62:418-423.

Shields, L.M., and L.W. Durrell. 1964. Algae in relation to soil fertility. Botanical Review 30: 92-128.

Starks, T.L., L.E. Shubert, and F.R. Trainor. 1981. Ecology of soil algae: a review. Phycologia 20(1):65-80.

St. Clair, L.L., and J.R. Johansen. 1993. Introduction to the symposium on soil crust communities. Great Basin Naturalist 53(1): 1-4.

St. Clair, L.L., J.R. Johansen, and B.L. Webb. 1986. Rapid stabilization of fire-disturbed sites using a soil crust slurry: inoculation studies. Reclamation and Revegetation Research 4: 261-269.

St. Clair, L.L., B.L. Webb, J.R. Johansen, and G.T. Nebeker. 1984. Cryptogamic soil crusts: enhancement of seedling establishment in disturbed and undisturbed areas. Reclamation and Revegetation Research 3: 129-136.

Tchan, Y.T. 1952. Fluorescence microscopy for the study of soil algae. Proceedings of the Linnean Society of New South Wales 77: 265-269.

Vollenweider, R.A. 1969. A manual on methods for measuring primary production in aquatic environments, including a chapter on bacteria. Pages 29-32 *in* R.A. Vollenweider, ed., Blackwell Scientific Publications, Oxford and Edinburgh.

West, N.E. 1990. Structure and function of microphytic soil crusts in wildland ecosystems of arid to semi-arid regions. Pages 179-223 in *Advances in Ecological Research Volume 20.* Academic Press Limited.

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